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COMPARATIVE STUDIES ON THE FUNCTION OF GILLS IN SUSPENSION FEEDING BIVALVES, WITH SPECIAL REFERENCE TO EFFECTS OF SEROTONIN

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An extensive literature deals with the function of the exposed gill or gill fragments of suspension-feeding bivalves in transporting water and in retaining and sorting particles suspended in the water (see Jørgensen, 1966). These studies have led to the view that normal feeding results from the largely independent activities performed by the three main ciliary systems on the gill filaments: the lateral cilia transport water, the latero-frontal cirri intercept and retain particles suspended in the water, and the frontal cilia sort and transport retained particles to ciliary tracts along the gill bases or the free margins of the demibranchs.

The function of the latero-frontal cirri in straining particles from the water passing through the interfilamentar spaces and in transferring them to the frontal ciliary tracts was studied in the mussel *Mytilus edulis* by Dral (1967), who concluded that the intercirral distances, 2–3 μm, determined how small particles could be efficiently retained. Measurements of the efficiency with which *Mytilus* and other bivalves clear suspended particles from the ambient water showed, however, that even smaller particles could be efficiently retained. The discrepancy between predicted and measured efficiencies of particle retention in *Mytilus* seemed to vanish when it was observed that the cirri are featherlike structures, composed of cilia of different lengths with distal ends branching off from the main stem of the cirrus at regular intervals of about one μm (Moore, 1971). It was assumed that these side branches of the cirri form the filter that is responsible for the retention of particles smaller than the intercirral spaces (Moore, 1971; Owen, 1974a, b).

However, other suspension feeders that possess gills with short (oysters) or undeveloped (scallops) latero-frontal cirri can also efficiently retain particles (Jørgensen and Goldberg, 1953; Haven and Morales-Alamo, 1970; Vahl, 1972a, b, 1973a, b). Moreover, the *Mytilus* gill may under circumstances become leaky even to large particles despite apparently normally beating latero-frontal cirri (Jørgensen, 1975). It was, therefore, found of interest to examine more closely how exposed gills and isolated gill fragments of various gill types transport water and deal with suspended particles, and especially to compare the efficiency with which particles are retained in relation to the size and function of the latero-frontal cirri.

Serotonin (5-HT) has been found to act in a cilio-excitatory way on the bivalve gill. It has been assumed that the serotonergic nerves demonstrated in gill filaments of several bivalves control ciliary activity, especially of the lateral cilia (Aiello, 1960, 1970; Gosselin, 1961; Paparo, 1972; Paparo and Finch, 1972; Stephano and Aiello, 1975). Less is known about the role that serotonergic innervation may play in controlling the activity of the latero-frontal cirri (or other ciliary systems of the gill). It was, therefore, investigated how 5-HT affected the function of the isolated gill in transporting water and retaining suspended particles.

Materials and Methods

The investigations were made at the Red Sea, Elat, Israel, in December 1974. The bivalves studied, all belonging to the epifauna, included *Anomia* and several species of oysters attached to anchored rafts, *Tridacna* and *Pteria* obtained from coral reefs, and one young pectinid, *Juxtamusium,* found in an oyster pond temporarily out of use.

The types of gills examined included homorhabdic, filibranch gills, *Anomia achaeus,* Gray 1849; heterorhabdic, plicate gills, *Juxtamusium maldivense* (E. A. Smith, 1903), *Pteria macroptera* (Lamarck, 1819), *Pycnodonte hyotis* (Linnaeus, 1758), and *Pycnodonte numisma* (Lamarck, 1819); and heterorhabdic, tubular gills, *Crassostrea cucullata* (von Born, 1778) = *Saccostrea cucullata* (see Ahmed, 1975), *Crassostrea lugubris* (Sowerby), *Tridacna maxima* (Röding, 1790), and *Tridacna squamosa,* Lamarck, 1819. The familial allocations of these bivalves are as follows: *Anomia* in the Anomiidae; *Juxtamusium* in the Pectinidae; *Pteria* in the Pteriidae; *Pycnodonte* in the Gryphaeidae (or in the Ostreidae); *Crassostrea* in the Ostreidae; and *Tridacna* in the Tridacnidae.

Observations on intact gills were made under a dissecting binocular microscope and on gill fragments under a monocular microscope, at magnifications up to 1000 times. Water currents produced by the gills or gill fragments were visualized by the addition of drops of a culture of the flagellate *Tetraselmis suecica* to the ambient water. The ellipsoid cells of *Tetraselmis* were about 12×8 μm, or more, in dimensions.

Observations started immediately after the gills had been exposed by removal of one valve and mantle. Fragments of gills were observed freshly, and at intervals, until they began to disintegrate, up to 3–4 days after they had been isolated from the bivalve. Between inspections, the preparations were placed in covered Petri dishes with sea water and kept at room temperature.

Recordings were made of gill movements and their types; movements of algal cells, and other particles suspended in water, in relation to the gill plicae and filaments, especially to what extent algae were retained by the gill or passed between the filaments; and activity of the various ciliary tracts, especially the latero-frontal cirri and the effect of 5-HT on their pattern of activity and rate of beat.

10^{-4} M 5-HT, dissolved in sea water, was usually added to the preparation to produce concentrations in the ambient medium of 10^{-5}–10^{-4} M.

Results

Gill movements

Two main types of gill movements can be distinguished: first, antero-posterior and dorso-ventral, intermittent contractions, which were usually more violent (convulsive) in intact gills than in gill fragments isolated from the gill bases; and secondly, concertina-like movements of plicae or filaments (Table I). Gills exhibited the greatest variation in the convulsive type of gill movements. The most violent contractions were observed in the gill of *Pteria macroptera,* which was also

TABLE I

Types of gill movements. 0 indicates movements which are weak or absent; +, moderate; and ++, strong. The asterisk indicates gill fragments appear permanently contracted.

Species	Phasic contractions		Concertina-like movements	Nature of filament interconnections
	Untreated	After 5-HT treatment		
Anomia achaeus	+		+	ciliary bridges
Juxtamusium maldivense	++		0–+	ciliary bridges
Pteria macroptera	++	0	+	ciliary bridges
Crassostrea cucullata	0		+	epithelium with ostia
Crassostrea lugubris	0		0–+	epithelium with ostia
Pycnodonte hyotis	++	0	+	tissue bridges
P. numisma	++	0	+	tissue bridges
Tridacna maxima	+*	+*		tissue bridges
T. squamosa	+*	+*		tissue bridges

highly sensitive to mechanical stimulation, as noticed by Atkins (1936, p. 275) in *Pteria hirundo*. In other species, such as the two oysters, *Crassostrea cucullata* and *Crassostrea lugubris,* both the intact gill and isolated fragments showed only slight overall contractions, the gill movements being mainly restricted to local contractions within individual plicae.

The violent gill contractions observed especially in *P. macroptera* and both species of *Pycnodonte* were caused by contractile elements running both in an antero-posterior and dorso-ventral direction within the lamellae of the demibranchs. The elements producing the antero-posterior contractions act through the filament bridges, which are ciliary in *Pteria*. The dorso-ventral contractions are due to intrafilamentar muscle fibers.

Addition of 5-HT to the medium in concentrations of 10^{-4}–10^{-5} M in most cases caused relaxation of contracted gills, and reduced or abolished the response to mechanical stimulation. Exceptions were the two species of *Tridacna*. Fragments of the small, compact gills in these species appeared slightly contracted during the three days of observation, and the preparations showed no clear reaction to 5-HT.

The slow concertina-like movements cause neighboring plicae, or filaments, to move alternatingly towards and away from each other. In the homorhabdic, filibranch gill lamellae, as in *Anomia*, the concertina-like movements are due to the activity of the cilia constituting the ciliary bridges, which connect the filaments. In the heterohabdic, plicate gills, concertina-like movements of the plicae are caused by the activity of muscle fibers present in the abfrontal tissue of the plicae. This is also true in species in which the filaments constituting the plicae are united by ciliary bridges (*Juxtamusium*, *Pteria*).

The concertina-like movements of plicae were observed in all plicate gills, except in *Tridacna*. The movements tended to be intermittent and varying in intensity. In *Juxtamusium* they could be correlated with the functional state of the water-transporting lateral cilia. When all lateral cilia of a plica were active, the plica became inflated, presumably due to the hydrostatic pressure produced by the water pressed into the intraplical space by the beating lateral cilia. In this functional state, movements of the plica were discontinued. At periods of arrest of the lateral cilia the plica collapsed, and movements of the plica were often resumed. Usually intermittent periods of activity and arrest of the lateral cilia and of concertina-like movements extended over several plicae of a gill lamella.

5-HT had no clear effect on the concertina-like movements of the gill plicae in most species. In *Juxtamusium*, however, addition of 5-HT stimulated the movements.

Particle movements and water currents

Exposed gills. When one valve and mantle were removed in the specimens of bivalves examined, and a drop of *Tetraselmis* culture was added to the water, algal cells could be observed to accelerate toward the gill surface, mostly to disappear between the filaments. This behavior of suspended algae, and other particles present in the medium, indicated that currents of water moved toward the gill surface and further between the filaments.

Some algae or particles were, however, retained on the surface of the gill and were carried toward the gill base or the ventral margin of the demibranchs (Table II). In *Anomia*, all particle transport on the gill surface was toward the ventral margin, along which further transport was aborad. The exposed gill in *Pteria* also transported retained particles ventrally. In this species, however, particles that arrived at the ventral margin were not transported further, but became engaged in a stationary, rotating movement. In other species, transport of particles on the gill surface was predominantly toward the gill bases, and further orally along the dorsal groove (*Juxtamusium*, *Pycnodonte*). In *Tridacna*, the inner demibranch transported particles toward the ventral margin and further orally along this margin; whereas the outer, smaller, demibranch, which lacks the ventral margin and its

TABLE II

Ciliary activities and effects of 5-HT on filaments of gill fragments. For frontal cilia, v represents ventrad; d, dorsad; 0, no or weak current; +, normal current. For lateral cilia, 0 represents no or only scattered activity; (+), extensive, but unstable metachronal activity; +, stable and slow metachronal activity; ++, stable and fast metachronal activity. For latero-frontal cirri, N represents normal beating; R, beating at reduced angle; S, beating stopped; H, cirri in horizontal position; O, in oblique position; and V, in vertical position.

Species	Length of specimens in cm	Direction and strength of frontal ciliary currents		Activity of lateral cilia		Predominant patterns of activity of latero-frontal cirri	
		Ordinary filaments	Principal filaments	Untreated	After 5-HT treatment	Untreated	After 5-HT treatment
Anomia achaeus	2.5	v +	—	+	++	—	—
Juxtamusium maldivense	1.8	v +	d +	++	++	—	—
Pteria macroptera	6.0	v 0	d +	(+)–+	++	—	—
Crassostrea cucullata	Adults	v +	d +	(+)–+	++	N	SV
Crassostrea lugubris	2–3	v +	d +	(+)	++	SH-SV, N	N-SV
Pycnodonte hyotis	4–5	v 0	d +	(+)	++	SO	SO
P. numisma	3–4	v 0	d +	(+)	++	SO	SV-R
Tridacna maxima	4.0	v +	v +	(+)	+	SH-R	R-SV
T. squamosa	5.0	v +	v +	0	0	SH-SV	SH-SV

ciliary tracts, transported particles toward the inner dorsal groove (Stasek, 1962).

Gill fragments. The particle movements and water currents in relation to the gill surface could be examined in greater detail on gill fragments observed under the monocular microscope.

The predominant directions in which added *Tetraselmis* cells moved close to the frontal surface of the gill filaments are shown in Table II. In all species, except *Tridacna,* particles in interplical grooves moved dorsally, whereas on the plical crests particles in most species moved toward the ventral margin. Generally, the *Tetraselmis* cells tended to become concentrated within the interplical grooves, when these were open. Moreover, particles moved at greater speed within the grooves than on the plical crests. In some species, particles on the plical crests moved only slowly, or not at all, *e.g.,* in *Pteria* and both species of *Pycnodonte.*

Particles in the bottom of the interplical grooves travelled dorsally in a current of water produced by the frontal cilia on the principal filaments, and sometimes also on the neighboring filaments (*Crassostrea cucullata*). Presumably, the particles were predominantly suspended in the water current and not carried directly by the frontal cilia. This was indicated by the pattern of movements of the algal cells, which often crossed the principal filament moving at a level above the tips of the cilia constituting the frontal tract. Often the algae left the main current and passed through the interfilamentar spaces, (*e.g., Juxtamusium, Pteria, Pycnodonte*), or through the ostia perforating the subfilamentar membrane (*Crassostrea*).

Also particles transported toward the ventral margin higher up the plical sides and on the crests were predominantly carried in water currents produced by the frontal cilia. Mostly, particles travelled over only short distances before they disappeared between the filaments or were carried down into the interplical groove to enter the dorsally-directed water current (*e.g., Juxtamusium, Pteria, Crassostrea*).

Particles penetrating the interfilamentar space often performed characteristic jumping movements before they disappeared.

In most species, addition of 5-HT to the medium enhanced the rate at which *Tetraselmis* cells moved toward the gill surface and along the interplical grooves. Concomitantly, a greater proportion of the algae passed between filaments, and jumping before passage became more frequent and vigorous.

Jumping of particles on the surface of the gills of bivalves has previously been described by Atkins (1936, p. 275), Galtsoff (1964, p. 136), and Jørgensen (1975, p. 216). Atkins suggests that the beating of long, stout cirri is responsible for the jumping, whereas Galtsoff states that the particles are discarded by the recovery stroke of the lateral cilia. More likely the jumping reflects passive movements of the particles in local water currents produced by the activity of the metachronally beating lateral cilia (Jørgensen, 1975).

Ciliary activity

Lateral cilia. The lateral cilia are the best studied of the ciliary tracts present on the filaments of suspension-feeding bivalves. The tracts of lateral cilia vary only little between species. On gill preparations the lateral cilia beat in a plane nearly at a right angle to the long axes of the gill filaments, the active stroke being toward the abfrontal surface of the filament. The cilia beat metachronally, the metachronal wave travelling at a right angle to the plane of the beat. The direction of the effective stroke is to the left of the direction of the wave, that is, laeoplectic metachronism (Knight-Jones, 1954; Aiello and Sleigh, 1972).

The lateral cilia continued their metachronal activity on the gill fragments of all species studied, up to 4 days after preparation in *Anomia*, and 2 to 3 days in most of the other species. Often, however, the frequency of beating decreased soon after preparation, and sometimes the activity became unstable, the beat ceasing intermittently over smaller or larger distances of single filaments, or larger areas of the gill surface. Addition of 5-HT typically restored rapid and stable beating of the lateral cilia (Table II). Exceptions were *Juxtamusium* and *Tridacna*. In intact gills and gill fragments of *Juxtamusium*, the lateral cilia continued beating rapidly, with intermittent periods of ciliary arrest locally. Addition of 5-HT made no visible change in this pattern of activity. An increase in frequency of beating might have been disclosed if the frequency had been accurately measured. In *Tridacna*, the lateral cilia were only moderately active, even after addition of 5-HT in concentrations up to about 10^{-4} M.

Latero-frontal cirri. As mentioned, the latero-frontal cirri have been ascribed a special function in straining particles from the water passing through the interfilamentar spaces of the gills in suspension feeding bivalves. Special attention was, therefore, paid to the shape and function of the latero-frontal cirri. Atkins (1938) described in detail the distribution and development of latero-frontal cirri in the various types of bivalve gills, but a comparative functional study is still lacking.

Typically, latero-frontal cirri beat in a plane at a right angle to the long axis of the gill filaments. In the transitional stage between a recovery stroke and the subsequent active stroke, latero-frontal cirri occupy a horizontal position in the plane of the frontal surface of the filaments. The angle of beat is approximately 90°, so that at the end of the active stroke the cirri are oriented vertically onto the gill surface. A possible straining function of the cirri must depend upon the extent to which the cirri span over the interfilamentar space when they are in their horizontal position. It was, therefore, recorded how far into the interfilamentar space the cirri were able to reach in the various species of bivalves examined.

Latero-frontal cirri could not be distinguished on the gill filaments of *Anomia*, *Juxtamusium* and *Pteria*, even at magnifications up to 1000 times. They were present in the remaining species, but varied in size between species. Also their pattern and degree of activity, as well as their reaction to stimulation with 5-HT varied (Table II).

Length of latero-frontal cirri. The latero-frontal cirri were medium-sized in *Crassostrea cucculata* and in the two species of *Pycnodonte*, being about 17 μm in length. In the horizontal position they reached into the interfilamentar space about as far as the tips of the lateral cilia when oriented vertically on the epithelial surface during their active stroke. In the relaxed condition of the plicae this was about half the distance to the middle of the interfilamentar space.

In *C. lugubris* the latero-frontal cirri were small and inconspicuous, being about 13 μm in length. At times they were difficult to see. In their horizontal position they protruded into the interfilamentar space only to the level of the profile of the metachronal wave represented by the lateral cilia during their recovery stroke. In the relaxed plicae only a minor part of the interfilamentar space was covered by the latero-frontal cirri.

Also the two species of *Tridacna* possessed small latero-frontal cirri, about 15 μm long. In these species, however, because of the narrow interfilamentar spaces, the tips of opposing cirri met in their horizontal position. As mentioned, the plicae of the gill fragments may have remained contracted during the whole period of observation of 2–3 days. It, therefore, remains to be ascertained whether the bridging of the interfilamentar spaces by the latero-frontal cirri represents the undisturbed condition in *Tridacna*.

The above description of the relations of the latero-frontal cirri to the interfilamentar space applies to the ordinary filaments in all species examined. It also applies to the principal filaments, except in the two species of *Pycnodonte* and in *C. lugubris*. In these the latero-frontal cirri on the principal filaments are situated on the frontal surface at a distance from the sides, so that the cirri do not reach the rounded edges of the broad principal filaments.

Activity of latero-frontal cirri. As indicated in Table II the latero-frontal cirri showed great variability in the degree of activity and in orientation of inactive cirri, as observed on nonstimulated isolated gill fragments. The cirri on the gill fragments of *Crassostrea cucullata* were most regular in behavior, practically all beating steadily at an angle of about 90°. On the gill fragments of the other species examined, the activity of the cirri was less regular. Mostly, the cirri were at rest occupying various positions between horizontal and vertical to the surface of the gill. In *Tridacna maxima,* the activity pattern tended to change with time. During the day of preparation, most cirri were at rest in the horizontal position. On the third day, beating at a small angle close to the vertical position predominated. In *T. squamosa,* most cirri were also at rest on the first day, but occupying all positions between the horizontal and vertical. Gill fragments of more specimens of the various species must be examined before it can be safely concluded that the patterns described are typical for the species.

In *Mytilus edulis* it was previously found that addition of 5-HT to the ambient medium in concentration of about 10^{-5} M caused regularly beating latero-frontal cirri to discontinue beating and to remain in the vertical position (Jørgensen, 1975). This reaction was also observed in *Crassostrea cucullata,* in which most latero-frontal cirri ceased beating and remained motionless in the vertical position when 5-HT was added to provide a concentration in the medium of 10^{-5}–10^{-4} M. However, the response was less complete than in *M. edulis,* some cirri continuing to beat sporadically, though often at a reduced angle. In other species, the response of the latero-frontal cirri to stimulation with 5-HT was less pronounced or almost lacking. However, the type of response was generally similar to that observed in *M. edulis* and *C. cucullata,* that is, reduction of the angle of beat or stoppage in a position oriented obliquely or vertically to the surface of the gill. *Crassostrea lugubris* seems to represent an extreme with respect to the response of the small latero-frontal cirri toward stimulation with 5-HT. In two out of three fragments observed, the cirri were at rest on the unstimulated preparation, oriented in positions between horizontal and vertical. In these preparations, addition of 5-HT to a concentration of 10^{-5}–10^{-4} M reestablished extensive beating of the cirri at angles of 90°. Increasing the concentration of 5-HT about 10^{-4} M reduced the number of beating cirri, and most of those brought to rest remained in the vertical

TABLE III

Effect of 5-HT on creeping rate of gill fragments of Pycnodonte numisma. *Figures indicate mean values in μm/sec; ranges are in parentheses. N is the number of readings.*

Preparation number	Concentration of 5-HT					
	0	N	10^{-6} M	N	10^{-5} M	N
1	320 (290–350)	3	160 (140–170)	4	130 (125, 130)	2
2	260	1	170	1	140	1
3	320 (290–360)	5	190 (180–220)	5	130 (100–170)	9

position. More experiments are needed to clarify the reactions of the laterofrontal cirri to 5-HT in different species of bivalves.

Frontal cilia. The activity of the frontal cilia was not studied systematically in the present investigation. General conclusions concerning their activity can, however, be drawn from the observations recorded above on the behavior of particles moving along the gill surface. These observations indicated that most frontal ciliary tracts continue beating unaffected by the isolation of the gill fragment from the rest of the body. In species with plicate gills this statement especially applies to the frontal ciliary tracts of filaments within the interplical grooves. On the filaments constituting the crests, particle transport, and consequently activity of frontal ciliary tracts, was in some species slow or absent (Table II).

It was noticed that gill fragments of *Pycnodonte numisma,* which exhibited only a weak particle transporting current along the plical crests toward the ventral margin, when placed in sea water in a Petri dish, exhibited creeping behavior more consistently than gill preparations from most other of the species examined. The gill fragments included the ventral margin, which constituted the trailing edge of the creeping fragment. Presumably, the movements were caused by the beating frontal cilia of the plical crests. It is suggested that the mechanical stimulation of cilia caused by their contact with the bottom of the dish activated the cilia. This activation continued for at least 24 hours, the period during which the gill fragments were observed to maintain their creeping activity with undiminished vigor.

In some preparations the rate of creeping was measured and the effect of 5-HT studied. It can be seen from Table III that 5-HT reduced the rate and that the reduction was stronger at a concentration in the ambient medium of 10^{-5} M than at 10^{-6} M.

Giant cirri. Giant cirri have been described on gill filaments of many species of bivalves, especially on the frontal and abfrontal surfaces of the filaments (see Atkins, 1936, 1937a). They were also occasionally noticed in the present study, but in *Tridacna maxima* they were far more numerous than in any of the other species examined. Many giant cirri were present, especially along the ventral margin of the inner demibranch and on the frontal surface of the filaments. The cirri were oriented vertically on the surface and were inactive most of the time. 5-HT in concentrations of 10^{-5}–10^{-4} M had no effect on the cirri, which remained motionless.

Mucus

Removal of one valve and mantle in order to expose the gill or excision of gill fragments stimulated moderate secretion of mucus on the surface of the gill. In the absence of further stimulation the secretion of mucus soon decreased to low levels. Only in *Tridacna*, mucus tended to accumulate on the gill surface. In the other species examined the frontal ciliary tracts immediately carried the mucus to the ventral margin or the dorsal groove. The intensity of mucous secretion could, therefore, be estimated from the amounts of mucus that accumulated at the cut ends of the ciliary tracts of the ventral margin and dorsal groove. 5-HT did not affect mucous secretion.

The role played by mucus in the retention and transport of particles by the bivalve gill has been much debated. It is, therefore, of interest to note that only a minor part of *Tetraselmis* cells retained by the intact gills or gill fragments of the bivalves examined became entrapped in mucus and were carried directly on the ciliary collecting tracts. The majority of retained algal cells, and other particles, were carried in the water currents above the ciliary tracts. These concentrated suspensions of algae could be observed to leave the gill fragments at the cut ends of the ciliary tracts of ventral margins and dorsal grooves, to redisperse in the ambient medium. The redispersed algal cells did not tend to adhere to each other, and they continued swimming normally by means of their flagella, indicating that the cells had not become smeared with mucus during the process of retention and transport by the gill. Algae that did become entangled in mucus accumulated at the cut ends of the ciliary tracts, being unable to become resuspended in the water.

Discussion

Observations made on exposed or isolated gills or gill fragments from various types of bivalve gills show that such preparations continue to transport water, but they only inefficiently retain particles suspended in the water. This is contrary to gills in intact, undisturbed bivalves, which retain particles efficiently down to sizes of a few μm in diameter (*Mytilus edulis*, Jørgensen and Goldberg, 1953; Vahl, 1972a; Jørgensen, 1975; *Crassostrea virginica*, Jørgensen and Goldberg, 1953; Haven and Morales-Alamo, 1970; *Cardium edule*, Vahl, 1973a; *Chlamys opercularis*, *C. islandica*, Vahl, 1972b, 1973b).

Gill filaments of bivalves receive serotonergic innervation (Paparo, 1972; Paparo and Finch, 1972), which seems to be cilio-excitatory (Aiello, 1960, 1970; Gosselin, 1961). Exogenous 5-HT may stimulate the rate of water transport through gill preparations, but the drug did not restore retentiveness of the gill. If anything, the gill became more leaky to 10–20 μm *Tetraselmis* cells added to the ambient medium, probably an effect secondary to an increased rate of water flow through the interfilamentar spaces.

MacGinitie concluded from studies published already in 1941 that only undisturbed suspension feeding bivalves feed normally. His observations had, however, little impact on subsequent studies on the function of the gill in feeding bivalves. Investigators have continued to examine mechanisms of particle transport and sorting on preparations, including bivalves with the gills exposed by removal of one valve or part of a valve and mantle, or isolated gill preparations (*e.g.*, Nelson, 1960; Stasek, 1962; Galtsoff, 1964; Morton, 1969; Fankboner, 1971; Narchi, 1972; Bernard, 1974).

One reason for the continued interpretation of observations on how particles are dealt with by exposed gills or by gill fragments in terms of normal feeding mechanisms is probably to be found in the apparent expediency in the functioning of such preparations. They continue performing complex activities, such as straining, sorting, and transporting particles, as described in detail in numerous species of bivalves, especially by Atkins (1936, 1937a, b, 1938). Less emphasis was placed on the observation, in most cases casually mentioned (Galtsoff, 1964; Bernard, 1974), that removal of merely part of one valve and mantle caused leakiness of the gill. Such preparations may survive in the laboratory for several months and appear to function normally (*Crassostrea gigas*, Bernard, 1974). From observations on three species of oysters with one mantle removed and kept in sea water from a circulation tank for several months, Nelson (1960) concluded that undisturbed oysters in clean water transport water through the gills without retaining suspended particles. Only addition of sufficient amounts of suspensions of carmine or phytoplankton induced retention of particles by causing contractions of ostia and filaments.

Experiments of the type made by Nelson, Galtsoff and Bernard illustrate how resistant bivalves can be toward mutilation with respect to ability to survive. This hardiness of bivalves toward adverse conditions has concealed that bivalves are also highly sensitive toward handling and changes in their normal environment. Presumably, one of the functions most easily impaired is the normal feeding activity of the gills.

It remains to be understood how the bivalve gill transports water at high rates and efficiently retains small particles. It has been suggested that the activity of the latero-frontal cirri is responsible for efficient retention (Dral, 1967; Moore, 1971; Owen, 1974a, b). This hypothesis cannot apply to species that lack latero-frontal cirri, such as *Anomia, Pteria* and pectinids (Vahl, 1973b).

According to MacGinitie, undisturbed bivalves produce while feeding a continuous sheet of mucus covering the gill surface and acting as a filter that strains even colloidal particles from the passing water (MacGinitie, 1941, 1945). There is no direct evidence to support MacGinitie's mucous sheet hypothesis. It seems to be inconsistent with the finding in several species of bivalves that the critical particle size for complete retention is about 2–4 μm in diameter. The role played by mucus in normal feeding remains to be elucidated. Secretion of mucus in amounts that may form sheets on the gill surface seems to serve cleaning purposes (Bernard, 1974; see also Jørgensen, 1966, for discussion.)

The finding that even slight disturbance of bivalves produces leaky gills suggests great lability of the functional organization of the ciliary system in the undisturbed feeding bivalve.

5-HT was observed to reduce the creeping rate of gill fragments of *Pycnodonte numisma*. Creeping rates of gill fragments have often been used to measure activity of the frontal cilia. However, Gosselin and O'Hara (1961) found that the effects of 5-HT on the rate at which the gill surface (*e.g.*, in *Mytilus edulis*) transported particles depended upon the size of the particles. 5-HT enhanced the rate of transport of small particles and reduced the rate of large particles. The authors explain these paradoxical effects of 5-HT by assuming that the motion of large particles along the frontal surfaces of the filaments is retarded by the perpendicular current of water generated by the lateral cilia, and that this retardation becomes more evident when the lateral cilia are stimulated by 5-HT. Jørgensen

(1975) observed that in the 5-HT stimulated gill of *Mytilus edulis* the latero-frontal cirri occupy positions vertical on the gill surface with their tips curved over the frontal surface of the filaments. In the experiments with the gill fragments of *Pycnodonte,* 5-HT both stimulated the activity of the lateral cilia and tended to arrest the latero-frontal cirri in a vertical position. Both effects may have contributed to the reduced creeping rates.

In conclusion, three functional states can be distinguished in the gills of suspension-feeding bivalves.

First, the *nonretentive* state is characteristic of disturbed animals and gill preparations. This state seems to have predominated in experiments on measurements of water transport by means of direct methods (Jørgensen, 1966). The functional significance of the state is not clear.

Secondly, *the cleaning state* is characterized by copious mucous secretion from the gill surface and activity of gill musculature. The state is typically elicited by high concentrations of suspended matter in the surrounding water. It has often been studied on exposed gills, whose function has been examined by means of thick suspensions added on to the gill surface.

Thirdly, *the feeding state* is characterized by high rates of water transport through highly retentive gills. This functional condition, which is presumably the phylogenetically youngest, is restricted to undisturbed bivalves. In studies on the functions of the bivalve gill, the feeding state has, therefore, mainly been approached indirectly.

The mechanisms by which the bivalve gills transport water at high rates and retain particles efficiently are not well understood. They may involve a functional integration of the different ciliary tracts on the frontal and lateral surfaces of the filaments.

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Summary

1. Observations were made on muscular and ciliary activity and particle transport and retention in intact gills and gill fragments of the suspension-feeding, epifaunal bivalves *Anomia achaeus* Gray, *Juxtamusium maldivense* (E. A. Smith), *Pteria macroptera* (Lamarck), *Pycnodonte hyotis* (L.), *P. numisma* (Lamarck), *Crassostrea cucullata* (von Born), *Crassostrea lugubris* (Sowerby), *Tridacna maxima* (Röding), and *T. squamosa* Lamarck.

2. Gill contractions were especially violent in *Pteria* and both species of *Pycnodonte*. 5-HT in concentrations of 10^{-4}–10^{-5} M caused relaxation of con-

tracted gills, and reduced or abolished the response to mechanical stimulation, except in *Tridacna*.

3. In *Juxtamusium*, the concertina-like movements of the gill plicae could be correlated with the functional state of the water-transporting lateral cilia. When all lateral cilia were active, and the plicae inflated, movements of the plicae were discontinued. At periods of arrest of the lateral cilia the plicae collapsed and resumed concertina-like movements. 5-HT had no clear effect on the concertina-like movements of the gill plicae, except in *Juxtamusium* in which addition of 5-HT to the ambient medium stimulated the movements.

4. Exposed gills or gill fragments of all the species examined continued to transport water, but only inefficiently retained particles, 10–20 μm *Tetraselmis* cells, added to the water. 5-HT enhanced the rate of water transport in most species by stimulating the activity of the lateral cilia, but reduced the ability of the gill to retain *Tetraselmis* cells. The cilio-excitatory nerve transmitter 5-HT of bivalve gill filaments thus did not restore normal feeding activity of the gill.

5. Latero-frontal cirri could not be distinguished on the gill filaments of *Anomia, Juxtamusium*, and *Pteria*. In *Crassostrea lugubris* they were small (*ca.* 13 μm, in length) and inconspicuous. They were about 15 μm long in the two species of *Tridacna*, and about 17 μm long in *Crassostrea cucullata* and in the two species of *Pycnodonte*, spanning about half of the interfilamentar space in relaxed plicae. The latero-frontal cirri varied greatly in activity and in orientation of inactive cirri. Also the effects of 5-HT were variable, but the drug tended to arrest the cirri in an erect position.

6. 5-HT reduced the creeping rate of gill fragments of *Pycnodonte numisma*.

7. The gill fragments secreted mucus at low rates even in dense suspensions of *Tetraselmis* cells. The majority of *Tetraselmis* cells that were retained and transported by the gill fragments remained free of mucus, to be redispersed in the medium when they arrived at cut ends of the particle transporting ciliary tracts along the gill bases or the ventral margins of the demibranchs.

8. It is concluded that the feeding state of the bivalve gill, which is characterized by high rates of water transport through highly retentive gills, is restricted to undisturbed, intact animals. The mechanisms of feeding, therefore, cannot be finally understood from studies on exposed gills or gill fragments. The physiological significance of the nonretentive gill of disturbed animals and gill preparations is not clear.

LITERATURE CITED

Ahmed, M., 1975. Speciation in living oysters. *Adv. Mar. Biol.*, **13**: 357–397.

Aiello, E. L., 1960. Factors affecting ciliary activity on the gill of the mussel *Mytilus edulis. Physiol. Zool.*, **33**: 120–135.

Aiello, E. L., 1970. Nervous and chemical stimulation of gill cilia in bivalve molluscs. *Physiol. Zool.*, **43**: 60–70.

Aiello, E., and M. A. Sleigh, 1972. The metachronal wave of lateral cilia of *Mytilus edulis. J. Cell Biol.*, **54**: 493–506.

Atkins, D., 1936. On the ciliary mechanisms and interrelationships of lamellibranchs. Part I. Some new observations on sorting mechanisms in certain lamellibranchs. *Quart. J. Microsc. Sci.*, **79**: 181–308.

Atkins, D., 1937a. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. Sorting devices on the gill. *Quart. J. Microsc. Sci.*, **79**: 339–373.

ATKINS, D., 1937b. On the ciliary mechanisms and interrelationships of lamellibranchs. Part III. Types of lamellibranch gills and their food currents. *Quart. J. Microsc. Sci.*, **79**: 375–421.

ATKINS, D., 1938. On the ciliary mechanisms and interrelationships of lamellibranchs. Part VII. Latero-frontal cilia of the gill filaments and their phylogenetic value. *Quart. J. Microsc. Sci.*, **80**: 345–436.

BERNARD, F. R., 1974. Particle sorting and labial palp function in the Pacific oyster *Crassostrea gigas* (Thunberg, 1795). *Biol. Bull.*, **146**: 1–10.

DRAL, A. D. G., 1967. The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel (*Mytilus edulis* L.). *Netherlands J. Sea Res.*, **3**: 391–422.

FANKBONER, P. V., 1971. The ciliary currents associated with feeding, digestion, and sediment removal in *Adula* (*Botula*) *falcata* Gould 1851. *Biol. Bull.*, **140**: 28–45.

GALTSOFF, P. S., 1964. The American oyster *Crassostrea virginica* Gmelin. *U. S. Fish Wildlife Serv. Fish. Bull.*, **64**: 1–480.

GOSSELIN, R. E., 1961. The cilio-excitatory activity of serotonin. *J. Cell. Comp. Physiol.*, **58**: 17–26.

GOSSELIN, R. E., AND G. O'HARA, 1961. An unsuspected source of error in studies of particle transport by lamellibranch gill cilia. *J. Cell. Comp. Physiol.*, **58**: 1–9.

HAVEN, D. S., AND R. MORALES-ALAMO, 1970. Filtration of particles from suspension by the American oyster *Crassostrea virginica*. *Biol. Bull.*, **139**: 248–264.

JØRGENSEN, C. B., 1966. *Biology of suspension feeding*. Pergamon Press, Oxford, 357 pp.

JØRGENSEN, C. B., 1975. On gill function in the mussel *Mytilus edulis* L. *Ophelia*, **13**: 187–232.

JØRGENSEN, C. B., AND E. D. GOLDBERG, 1953. Particle filtration in some ascidians and lamellibranchs. *Biol. Bull.*, **105**: 477–489.

KNIGHT-JONES, E. W., 1954. Relations between metachronism and the direction of ciliary beat in Metazoa. *Quart. J. Microsc. Sci.*, **95**: 503–521.

MACGINITIE, G. E., 1941. On the method of feeding of four pelecypods. *Biol. Bull.*, **80**: 18–25.

MACGINITIE, G. E., 1945. The size of the mesh openings in mucous feeding nets of marine animals. *Biol. Bull.*, **88**: 107–111.

MOORE, H. J., 1971. The structure of the latero-frontal cirri on the gills of certain lamellibranch molluscs and their role in suspension feeding. *Mar. Biol.*, **11**: 23–27.

MORTON, B., 1969. Studies of the biology of *Dreissena polymorpha* Pall. 1. General anatomy and morphology. *Proc. Malacol. Soc. London*, **38**: 301–321.

NARCHI, W., 1972. On the biology of *Iphigenia brasiliensis* Lamarck 1818 (Bivalvia, Donacidae). *Proc. Malacol. Soc. London*, **40**: 79–91.

NELSON, T. C., 1960. The feeding mechanism of the oyster II. On the gills and palps of *Ostrea edulis*, *Crassostrea virginica* and *C. angulata*. *J. Morphol.*, **107**: 163–203.

OWEN, G., 1974a. Feeding and digestion in the Bivalvia. *Advan. Comp. Physiol. Biochem.*, **5**: 1–35.

OWEN, G., 1974b. Studies on the gill of *Mytilus edulis:* the eulatero-frontal cirri. *Proc. Roy. Soc. London Ser. B.*, **187**: 83–91.

PAPARO, A., 1972. Innervation of the lateral cilia in the mussel *Mytilus edulis* L. *Biol. Bull.*, **143**: 592–604.

PAPARO, A., AND G. E. FINCH, 1972. Catecholamine localization, content, and metabolism in the gill of two lamellibranch molluscs. *Comp. Gen. Pharmacol.*, **3**: 303–309.

STASEK, C. R., 1962. The form, growth and evolution of the *Tridacnidae* (Giant Clams). *Arch. Zool. Exp. Gén.*, **101**: 1–40.

STEPHANO, G. B., AND E. AIELLO, 1975. Histofluorescent localization of serotonin and dopamine in the nervous system and gill of *Mytilus edulis* (Bivalvia). *Biol. Bull.*, **148**: 141–156.

VAHL, O., 1972a. Efficiency of particle retention in *Mytilus edulis* L. *Ophelia*, **10**: 17–25.

VAHL, O., 1972b. Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* (L.) (Bivalvia). *Ophelia*, **10**: 67–74.

VAHL, O., 1973a. Porosity of the gill, oxygen consumption and pumping rate in *Cardium edule* (L.) (Bivalvia). *Ophelia*, **10**: 109–118.

VAHL, O., 1973b. Efficiency of particle retention in *Chlamys islandica* (O. F. Müller). *Astarte*, **6**: 21–25.